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The

Journal of Infectious Diseases

FOUNDED BY THE MEMORIAL INSTITUTE FOR INFECTIOUS DISEASES

VOL. II *November 25, 1905* NO. 4

PUBLICATIONS FROM THE NEW YORK STATE CANCER LABORATORY. NO. 30.*
H. R. Gaylord, G. H. A. Clowes, G. N. Calkins.

SOME ARTEFACTS IN MOUSE CARCINOMA.

G. N. CALKINS AND G. H. A. CLOWES.

THE present paper deals with certain cell inclusions in mouse carcinoma, inclusions due to technical errors, but offering some evidence of an interesting type of intracellular necrosis.

The history of cell inclusions in carcinoma clearly shows that products of necrosis have been occasionally mistaken for parasites of one kind or another, and the possibility—especially after fixation with any mixture containing salts of mercury—of similarly mistaking artefacts is apparent to all laboratory workers.

The difficulty in removing precipitates from Zenker-fixed material is generally recognized, and treatment in bulk with iodized alcohol (often for weeks), is advised in most laboratory manuals, and for large pieces, subsequent treatment of paraffin sections is recommended. Rawitz's *Leitfaden für histologische Untersuchungen* advises treatment of pieces fixed in Zenker with "70 per cent alcohol with enough tincture of iodine added to give it a port wine color, the iodine and alcohol to be changed until the color no longer disappears" (1895, p. 17). Mercier[1] recommends 10–15 days at least in iodized 90 per cent alcohol and advises the use of clear tincture of iodine or strong concentrated iodized alcohol.

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* Received for publication July 20, 1905.

[1] *Zeit. für w. Mik.*, 1894, 11, p. 471.

The inclusions that we have to describe are not affected perceptibly by such treatment. The usual treatment of material in this laboratory is the same as that recommended in the majority of laboratory manuals, and the usual "mercury" deposits are frequently seen and recognized as such. In the series of mouse experiments with which the State Cancer Laboratory has been occupied for more than a year, such inclusions have been seen in the cancer tissues and passed over without much thought. In one case, however, the tumor when partially grown was stirred up thoroughly with a needle and then left for further developments. After some days it was observed that the tumor had grown smaller and the mouse showed signs of spontaneous recovery. The tumor was then removed and hardened in Zenker's fluid, treated as usual with iodized alcohol, embedded in paraffin, sectioned and stained according to the usual methods. The most wonderful variety of crystals and deposits, both intra and intercellular, were found in these sections, some comparatively large, others exquisitely minute.

The most common type of crystalline deposit in this tumor is comparatively small, dense, and nearly homogeneous, and with clear evidence of radiate structure usually with an eccentric focus. In other forms the radii are more marked, clearly defined, and the entire structure is fan-shaped (Plate 17, Figs. 1 and 4). In still other cases this fan structure is elaborated in much greater detail and many of the radii are carried out beyond the periphery as elongate, filamentous processes (Plate 18, Fig. 1). The ends of these processes bear local, cylindrical or sausage-shaped thickenings which give to the structure a striking resemblance to a fungus-growth.

In other cases of intercellular inclusions the crystallization does not result in uniform, definite masses, but in filaments without the dense basal portion. Such structures may start from the periphery of a hypertrophied cell, in which irregular masses of the deposit and the degenerated nucleus alone remain; while at the ends of the filaments again, are the enlarged, clublike, cylindrical masses (Plate 18, Fig. 2).

In still other cases the type of the crystallization differs from both of the above, resembling more than anything else the plan of structure of hoar frost on glass. Minute crystalline masses are attached end to end and a running crystalline structure results which may extend over an area of at least one-half a square millimeter.

The intracellular and intranuclear inclusions are even more remarkable than the intercellular. For convenience they may be distinguished as the "cytoplasmic" and the "nuclear" forms. The cytoplasmic forms are always in vacuoles which vary in size from minute vesicles to great intracellular spaces considerably larger than the nucleus. These small vesicles are seen in the vicinity of the nucleus and usually in the minute depression formed by the crescent-shaped nucleus (Plate 18, Figs. 6–10). Within the vacuoles are many curious, separate, filiform structures with characteristic spherical swellings at the ends (Plate 17, Figs. 5 and 6; Plate 18, Figs. 9 and 10). These filaments are usually single and separate, but in some cases they form a botryoidal group of characteristic arrangement (Plate 18, Fig. 8). Occasionally there is a central mass with irregular radiating branches (Plate 18, Fig. 13), while in a few cases the radiating structure, central mass, and knobbed radii furnish an intracellular parallel to the larger intercellular fan-shaped structures (Plate 18, Fig. 12).

Even more remarkable are the intranuclear filaments and knobs. Inside the nucleus there are minute masses similar in size and shape to the swollen ends of the radiating filaments (Plate 18, Fig. 5); or larger, swollen masses with irregular protuberances of variable length (Plate 18, Figs. 4 and 5), or finally, there may be long filaments with knobs on the ends similar to the knobbed filaments of the cytoplasmic forms (Plate 18, Fig. 3; Plate 17, Fig 3).

Attempts to stain these various structures are not successful; in some cases the structures have a faint homogeneous color which cannot be interpreted as a true staining reaction. The filaments and knobs are never stained even as much as this, although blackened by treatment with ammonium sulphide. Usually they present the same glassy or glairy appearance that characterizes fat under the microscope, and this appearance is more definite in the terminal swellings than in the substance of the filaments or the rays of the fan structure.

Unfortunately, on account of the limited amount of material at our disposal, it was impossible to make a thorough chemical investigation of these products. From micro-chemical tests however, it was found:

That a prolonged treatment with cold Lugol's solution removes all the bodies referred to above, a slight, amorphous residue being left within a few of the vacuoles.

That a short treatment with Lugol's solution leads to partial removal of the crystals, especially the large, extracellular, fan-shaped variety.

That treatment of the sections with dilute sulphuretted hydrogen or ammonium sulphide leads to a distinct blackening, the intensity of which is dependent upon the length of time that the sections are exposed to the reagent and the concentration of the latter. Sections so treated, when subsequently exposed to Lugol's solution at 50°, are entirely cleared in the course of half an hour.

These tests warrant the conclusion that the semi-crystalline compounds consist, at least in part, of mercury salts derived from the hardening agent. It should however be noted that whilst the large fan-shaped bodies blacken intensely under the influence of ammonium sulphide, the smaller intracellular and intranuclear bodies are blackened to a less marked extent and the fine filaments to which knobs are attached (Plate 18, Figs. 6–10), are apparently influenced very slightly, if at all.

The solubility of the various types of inclusions was tested in a series of organic solvents. Alcohol exerts very little influence in the cold, but at 50° for half an hour sections are entirely cleared of all inclusions, merely a slight residue being observed in some of the vacuoles. Ether, acetone, and admixtures of alcohol, ether, and acetone exert a similar, though possibly less marked, solvent action than does alcohol alone. Petroleum-ether and xylol produce very little effect, even at 50°. Chloroform apparently exerts more action than the latter and less than the former group of reagents. These results, when taken with the fact that salts of mercury are far more soluble at high temperatures in organic solvents than at normal temperatures, further justify the conclusion that the structures described above consist, at least in part, of salts of mercury.

To summarize the above results of chemical tests it may be said that mercury is unquestionably present in larger or smaller quantities in all the inclusions, both intra and intercellular. From the behavior of the crystals, especially the intracellular and intranuclear varieties, toward ammonium sulphide and other reagents it appears possible that the mercury salt is either deposited upon some product of necrosis of a fatty nature, or intimately associated with the latter in the form of a chemical compound. It has been found impossible to reproduce such forms from normal tissue or by means of a hardening agent admixed with various known products of decomposition. If any peculiar products of necrosis are associated with the salts of mercury in the inclusions referred to, the readiness with which they are removed by hot organic solvents and their behavior toward Lugol's solution and other reagents, as well as their lack of any characteristic micro-chemical reactions would lead us to suppose that they are of a fatty nature. In any case, it must be said that the figures observed resemble very closely the various forms assumed by fatty acids and fatty acid compounds when admixed with impurities.

While these deposits are to be interpreted as artefacts, the fact must not be overlooked that something of an unusual nature is present in these cancer cells and tissues, upon which the salts of the fixing agent work in forming the deposits of various kinds. The morphological evidence supports the view that necrosis of a marked type is in progress in these cancer cells; a necrosis beginning with cytoplasmic degeneration in the immediate vicinity of the nucleus and ending in great, hypertrophied and highly vacuolated, degenerated cells. The earliest morphological evidence of such degeneration is a minute vacuole in an otherwise normal cell (Plate 18, Fig. 6). It is conceivable that the material of this vacuolated region is metamorphosed into a substance upon which the salts of the Zenker fixing agent act to form the curious knobbed filaments. The necrotic cytoplasmic region increases in volume with further degeneration through continued action of the disease, and the fixing material acting on this larger mass produces an increased number of knobbed filaments in a greater cytoplasmic vacuole (Plate 18, Figs. 7–10). The ultimate result of this degenerating change is a huge cell skeleton consisting of a well filled out cell membrane, degenerated nucleus, and irregularly distributed material where the cytoplasm had been The fixing agent, acting on this stage of necrosis, gives few or none of the intracellular knobbed filaments, but, radiating out from the membrane into the surrounding intercellular spaces, may be found masses of such filaments with the characteristic terminal knobs (Plate 18, Fig. 2). From such evidence it might be inferred that the necrotic material produced in the degenerating cell had exuded through the cell membrane and was in the course of a general distribution when combined with the salts of the fixing agent. In still other regions the direct connection between the degenerated cell and the deposits is not so close, but throughout the immediate environment may be found the characteristic crystalline aggregates described above as a "running crystalline structure."

If our line of reasoning is correct, it appears that we have in this method of treating cancer tissues, i. e., by the creation of artefacts, a rough means of following morphologically the course of this particular type of degenerative change and the distribution of its products.

From a purely technical point of view, on the other hand, these results point out, in the clearest manner, the need of extreme caution in dealing with Zenker-fixed material. The ordinary precautions advised in every laboratory guide-book are inadequate, and more drastic measures must be employed to clear the tissues and cells of foreign bodies. For this we recommend treatment of paraffin sections for at least 30 minutes with Lugol's solution (potassium iodide plus iodine in water), or treatment of paraffin sections for about two hours with alcohol at a temperature of 50°.

BUFFALO, June 1905.

PLATE 17.

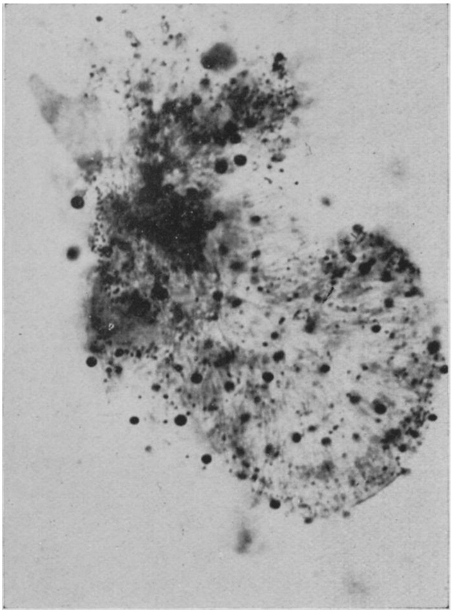


Fig. 1.

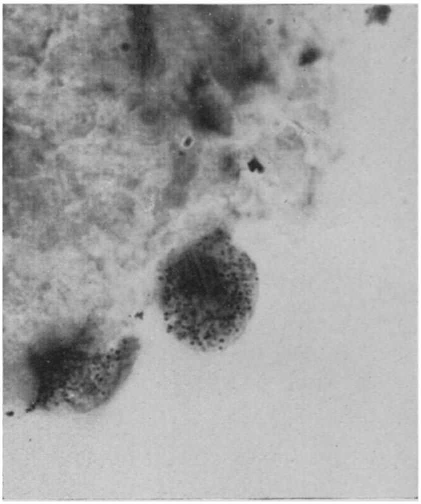


Fig. 2.

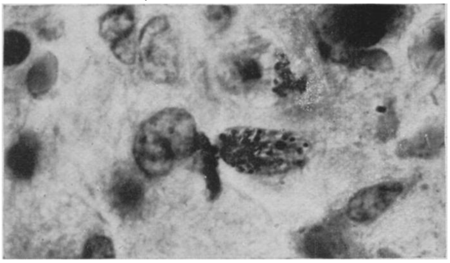


Fig. 3.

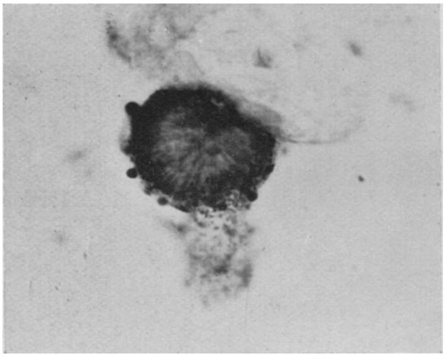


Fig. 4.

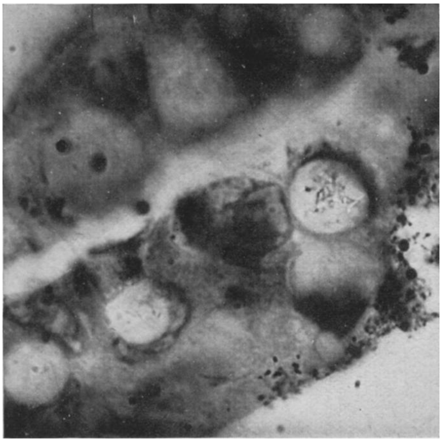


Fig. 5.

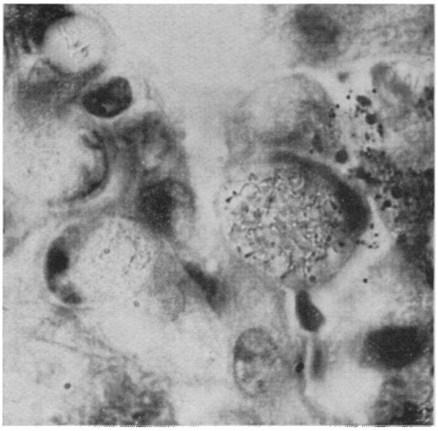
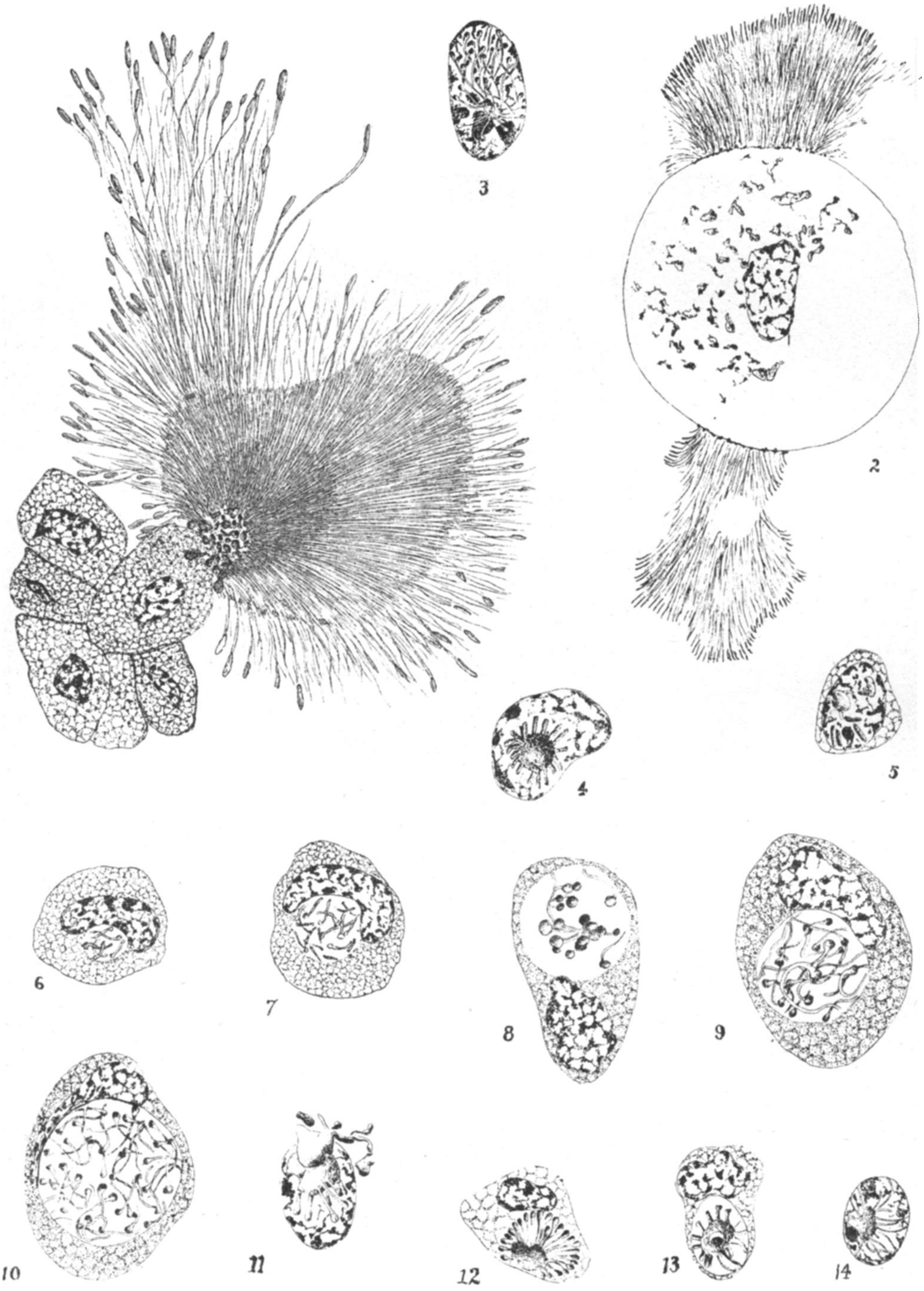


Fig. 6.

PLATE 18.



G. N. Calkins, del.

SOME ARTEFACTS in MOUSE CARCINOMA

DESCRIPTION OF PLATES.

PLATE 17.

(Microphotographs × 1000.)

FIGS. 1 and 4.—Microphotograph of small and large intercellular crystalline deposits, with radiating structure and granules of mercury.

FIG. 2.—Microphotograph of similar inclusion without radiate structure.

FIG. 3.—Microphotograph of nucleus with inclusions (Cf. Plate 18, Fig. 3).

FIG. 5.—Microphotograph of smaller vacuoles and crescentic nuclei.

FIG. 6.—Microphotograph of very large intracellular vacuole, crescentic nucleus and intra-vacuolar deposits.

PLATE 18.

(Camera lucida drawings from permanent preparations.)

FIG. 1.—Large intercellular crystalline deposit with radiate structure; the rays extending beyond the periphery are provided with terminal thickenings. × 1800.

FIG. 2.—Degenerate cell with remains of nucleus and flocculent deposit in place of the cytoplasm. From the cell periphery radiating fibers with terminal thickenings reach out into surrounding spaces. × 1000.

FIG. 3.—Nucleus with intranuclear deposits in fiber-form and with terminal knobs. × 1800.

FIG. 4.—Intranuclear deposit. × 1800.

FIG. 5.—Intranuclear deposits of variable size. × 1800.

FIG. 6.—Epithelial cell with beginning of vacuolar degeneration in the depression formed by the crescentic nucleus, and with intravacuolar inclusions. × 1800.

FIGS. 7, 8, 9, and 10.—Epithelial cells with vacuoles of increasing size and with accumulating, characteristic, knobbed inclusions. (Cf. Plate 17, Figs. 5 and 6.) × 1800.

FIGS. 11, 12, 13, and 14, various forms of intracellular and intranuclear deposits. × 1800.